Position Paper:

The Toxic Effects of Chronic Exposures to Low Levels of Carbon Dioxide

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REPORT NUMBER 973

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SUMMARY PAGE

PROBLEM

Representatives of various bureaus of the U. S. Navy occasionally consult scientists at NSMRL regarding safe limits of exposure to carbon dioxide during submarine patrols. Of particular concern recently has been the definition of the highest concentration of carbon dioxide which can be safely inhaled during continuous exposures for 30 to 90 days. This concern can be restated in more practical terms: "Are the inspired levels of CO_2 aboard Naval nuclear submarines toxic for humans who are continuously exposed for 90 days?"

FINDINGS

The responses of the human's organ systems were classified into primary and secondary responses to chronic, low-level, carbon dioxide exposures. Neither type of response represented a toxic effect.

APPLICATIONS

The position of this Laboratory is that it is presently unnecessary to conduct further investigations of human tolerance for CO_2 aboard submarines. Future needs of the Navy may, however, require research into the undesirable effects of chronic, low-level CO_2. The financial requirements for supporting such work will be extremely high.

ADMINISTRATIVE INFORMATION

This report was submitted on 28 December 1981. Following review it was approved for publication on 19 January 1982. It has been designated as NavSubMedRschLab Report Number 973.

PUBLISHED BY THE NAVAL SUBMARINE MEDICAL RESEARCH LABORATORY

ABSTRACT

Numerous patrol studies have provided a sizable data base of levels of CO_2 exposure and health problems encountered by Naval nuclear submariners. Humans have been exposed to a variety of concentrations of CO_2 for chronic periods of time aboard submarines, as well as in laboratory environments. Mean data, $^{P}I_{CO_2}$ = 10 torr and 40 days, collectively represent this wide variety of CO_2 exposures. >Physiological responses to the CO_2 environment were repeatedly documented, but toxic effects were not apparent.< Human exposures were safely conducted in atmospheres containing up to 5 torr CO_2, for up to 90 days. Such exposures are therefore considered safe at this time.

Animal studies showed that chronic, low-level, CO_2 exposures caused changes of the bone content of CO_2, the bone content of calcium, the soft tissue content of calcium, and histological changes of the lung. These changes were found to be completely reversible upon discontinuance of the animal's exposure to CO_2. >The extrapolation of the animal data to humans is a current problem in the advancement of our understanding of the effects of CO_2 on humans.<

There are considerable technical and financial impediments to conducting fruitful investigations of the effects of chronic exposure to low-levels of CO_2. The accurate maintenance of the required concentrations of CO_2 in the investigated environment is a costly endeavor. Many of the measurements of the organism's responses to CO_2 are difficult and insensitive and therefore currently limit low-level CO_2 research efforts. There are other significant factors which can modify an organism's responses to low-level CO_2. Diet, levels of exercise during confinement, trace contaminants, and time are important modifiers of any organism's response to CO_2. Failure to control these factors may result in false interpretations of the data from the experiments.

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TERMS

Acclimatization to CO_2 - The adaptive changes in response to deviations from normal $PaCO_2$, which have persisted for days (40).

Adaptation to CO_2 - The time to compensation of respiratory acidosis (4).

Chronic, low-level exposure to carbon dioxide - The continuous exposure to 2% (15 torr) or less of CO_2 for longer than 72 hours (10).

Gas Stores - The gases that may be found in the body (11).

Hypercapnia - Excess of carbon dioxide in the blood.

Hypercarbia - See hypercapnia.

Primary Effects of CO_2 - The changes of CO_2 stores, pulmonary function, blood gases, and acid-base balance during exposures to increased levels of CO_2.

Respiratory Acidosis - A failure of the pulmonary excretion of CO_2 at a rate equal to the rate of production (gain) of CO_2; and with the elevation of arterial P_{CO_2}, elevation of the arterial content of $H_{CO_3^-}$, and decline of the arterial pH. The cause can be either inadequate ventilation or CO_2 inhalation (15).

Secondary Effects of CO_2 - Changes of body functions other than those defined under "primary effects of CO_2".

Toxic Gas - a gas which is poisonous to the body (please refer to Chapter 7 for application of this term to CO_2).

ABBREVIATIONS

CO_2 = carbon dioxide

F_{ICO_2} = Fraction of carbon dioxide in the inspired air

$[HCO_3^-]$ = The concentration of bicarbonate ion

HCO_3^- = The bicarbonate ion

N = Number of crewmembers in the submarine

P_{ACO_2} = The partial pressure of carbon dioxide in alveolar gas

$PaCO_2$ = The arterial carbon dioxide tension

P_{CO_2} = The partial pressure of carbon dioxide

P_{ICO_2} = The partial pressure of inspired carbon dioxide

% CO_2 = Percentage of CO_2 in the submarine's atmosphere

SCFH = Standard cubic feet per hour

t = Time in hours

torr = Unit of pressure

torr-days = The product of inspired partial pressure of carbon dioxide and days spent exposed to carbon dioxide

V = floodable volume of the submarine, in cubic feet

HISTORICAL ASPECTS

Known Toxic Effects

High levels of CO_2 can exert a toxic effect on humans. Indeed, a human death was considered to possibly result from an acute exposure to 2% CO_2 (66) although an overpowering number of reports of similar CO_2 exposures would argue against it (61, 4, 26).

The experimental induction of acute respiratory acidosis results in the accumulation of carbonic acid until the reestablishment of CO_2 equilibrium. The content of HCO_3^- rises in the extracellular fluid. The accumulation of H^+ in the extracellular fluid is partially reduced by exchanges with Na^+ and K^+ from the bone and cells. Plasma proteins and hemoglobin act as buffers, as well as phosphates. The new steady state is characterized by a reduced arterial pH, a reduction of the ratio of $[HCO_3^-]/P_{CO_2}$, and presumably a higher cellular P_{CO_2}. The skeletal muscle pH is acidic (15, 21). Patients with chronic obstructive pulmonary disease have high values for their P_{ACO_2} (70 to 80 torr) due to inadequacy of their alveolar ventilation. Their chronic respiratory acidosis is not severe and does not require acid-base therapy. Three to 4 milliequivalents of $H_{CO_3}^-$ per liter are reabsorbed by the nephron for each 10 torr increment of P_aCO_2 in uncomplicated, chronic, respiratory acidosis. Hence, the change of pH is minimized by buffer supplied from the kidney. Severe deviations of pH can impair the functions of the vital tissues, namely the brain and heart (16). The electroencephalogram (EEG) undergoes a triphasic response as the fraction of inspired CO_2 rises from below 10% to above 40%. Concentrations up to 10% can depress the excitability of the cerebral cortex. Associated with this are hyperpolarization and accumulation of hydrogen ions within the neurons. The cortex progresses to an excitable state when the fraction of inspired CO_2 progresses toward 40%. The subcortical centers may spontaneously elicit seizures. The neurons are severely acidotic and there is a reduction of the total content of neuronal ions. The level of GABA is inversely correlated with the degree of brain excitability. Hyperexcitability can stimulate the release of catecholamines and cortisol (14). The tremendous decrement of cardiac muscle pH by 0.5 pH units results in 50% decrement of the force of contraction, and bradycardia, of the isolated heart. Inhalation of elevated levels of CO_2 may enhance the cardiac output by enhancement of the venous return or activation of the sympathetic nervous system. The postulated mechanism for enhancement of the venous return is the greater activity of the "respiratory pump" from chemo-stimulation of the respiratory control mechanism. Both the heart rate and arterial blood pressure rise in normal men when their P_{ACO_2} rises from 40 to 85 torr. Surgical anesthesia may result in transient elevations of P_{CO_2} up to 140 torr. Such elevations are well tolerated, the apparent danger being hyperventilatory hypotension. Ventricular arrhythmias may occur during hypercarbia or the correction of hypercarbia (17).

In view of the known toxic effects of high levels of CO_2 for brief exposures, a diversity of administrators and scientists have been concerned with the potential for toxic effects from prolonged exposures of humans to low concentrations of carbon dioxide

in the breathing medium. For example, the exploration of space requires that man live in a protective capsule for months while maintaining high standards of task performance and vigilance. Since high concentrations of CO_2 can degrade performance, the space officials need to know the essential requirements for cleansing the capsule of CO_2 in order to design an optimal CO_2-scrubbing apparatus capable of accompanying the astronauts into outer space.

History of CO_2 Research U. S. Navy

The U. S. Navy has had an identical problem with the encapsulated environments of its submarine force. The problem was formally recognized in 1914 when the U.S. Navy began serious study of the possibility of air purification of submarine atmospheres during submergences. For many years thereafter primary supportive effort was devoted to emergency removal of CO_2 because compressed air supplied the necessary oxygen. In 1929, and during the next 25 years, these significant developments were made at the Naval Research Laboratory (NRL):

(a) Anhydrous lithium hydroxide was developed for the emergency removal of CO_2 during long submergences.
(b) Self-contained oxygen rebreathers became standard throughout naval vessels. Potassium superoxide (KO_2) released oxygen for breathing and the alkaline residue absorbed CO_2.
(c) Chlorate candles were developed as a convenient source of oxygen for supplemental or emergency use.

It was recognized in the early 1950's that a whole new dimension in atmosphere life support systems would be necessary to enable nuclear-powered submarines to reach their potential or prolonged, uninterrupted submergences. Their submergence limitation would not be based on availability of underwater power but by the capability to maintain adequate life support for the crew. The nuclear submarine fleet today is capable of long submerged operations in safety and comfort for the crew.

Analytical data obtained by NRL chemists during early submergences of the first nuclear-powered submarines showed the atmospheres to contain many organic contaminants. Based on sampling and analysis, early nuclear submarine atmospheres contained O_2 19-21% and CO_2 0.1-1.3%. As individual contaminants were identified and their concentrations in the atmosphere established, the Navy Bureau of Medicine and Surgery had to consider their potential toxic effects and establish threshold limits for certain compounds. Human exposures are continuous for 24 hours a day for weeks on end in the sealed environment of the submarine. This is in marked contrast to industrial experience where exposure is 8 hours a day, 5 days a week. Therefore, the allowable limits had to be made much lower than those in the more conventional work place. For CO_2, as more was learned about possible effects, the allowable limits have been set lower several times, requiring changes in equipment or its operation.

An absolutely essential requirement in maintaining life under prolonged submergences is continuous removal of CO_2. The standard equipment used for this purpose is the MEA Scrubber. The CO_2 is stripped from the atmosphere by absorption in an aqueous solution of monoethanolamine (MEA) at room temperature. The CO_2 is released from the MEA solution by

passing it continuously through a heated chamber, and the concentrated CO_2 gas is pumped overboard. The atmospheric level of CO_2 is maintained at about 1.0% or less, which is considerably higher than the ambient concentration of a normal atmosphere (0.03%). However, crews have lived continuously with the higher concentrations of CO_2 for long periods without apparent ill effects. This factor has been under nearly constant study and review by the Bureau of Medicine and Surgery with no hard evidence of even minimal toxic effects. The emergency system for removal depends on alkaline absorption in a device in which the submarine atmosphere is blown through canisters of dry LiOH (1).

Respiration accounts for most of the carbon dioxide generated in a submarine. The concentration of the CO_2 varies considerably within permissible limits when the CO_2 scrubbers are working. Should the CO_2 scrubbers be rendered inoperable, intentionally or otherwise, the rise of the % CO_2 in the submarine can be calculated as follows:

$$\% \ CO_2 = 0.03 + \frac{85 \ t}{V/N}$$

Figure 1 represents the computed rate of rise of CO_2 in the atmosphere and can be used operationally to relate CO_2 scrubber function to safe limits of CO_2 tolerance.

The effects of short-term exposures to CO_2, summarized in figure 2, represents the U. S. Navy's official judgment on the hazards of CO_2 (3). Note there are extremes ranging from irreversible response to apparently absent responses. Apparently, no such chart exists for responses to long-term CO_2 exposures. The following policies have been reached regarding long-time CO_2 effects:

(a) 0.5 percent to 0.8 percent: There is probably no significant effect on the body.
(b) 0.8 percent to 3.0 percent: Prolonged exposure could induce impairment of mental functions and slowing of physical activities.
(c) 3.0 percent and above: Further impairment of mental functions and slowing of physical activities occur.

Prolonged exposure to elevated levels of CO_2 in the ambient air has been a major topic of study in submarine medicine and physiology for the past 35 years. The first significant studies in chronic CO_2 toxicity in man were done in two U. S. Navy laboratories. These studies were carried out under A. R. Behnke at the Naval Medical Research Institute (NMRI) and C. W. Shilling at the Naval Submarine Medical Research Laboratory (NSMRL). In determining the limits for increased CO_2 and lowered O_2 during emergency conditions aboard a submarine, the group at NMRI found that human subjects could tolerate up to 5% (38 torr) CO_2 and 12-13% (91-99 torr) O_2 for a period of 72 hours. Twenty percent of the men complained about headaches and occasional nausea while demonstrating some impairment in specific motor performance. The conclusion drawn from the experiment was that despite crew discomfort, the safety of a submarine need not be jeopardized. The group at NSMRL also demonstrated that 68 hours of prolonged exposure to 3% (21 torr) CO_2 and 17% (130 torr) O_2 impaired performance. Schaefer found that exposure to 3% (21 torr) CO_2 in air caused
(a) an attenuation of the ventilatory CO_2 response after 24 hours, and
(b) a decrease of the slope of CO_2 tolerance curve after 5-6 days. Following an initial drop, the bicarbonate level in the blood sufficiently increased after 3 days

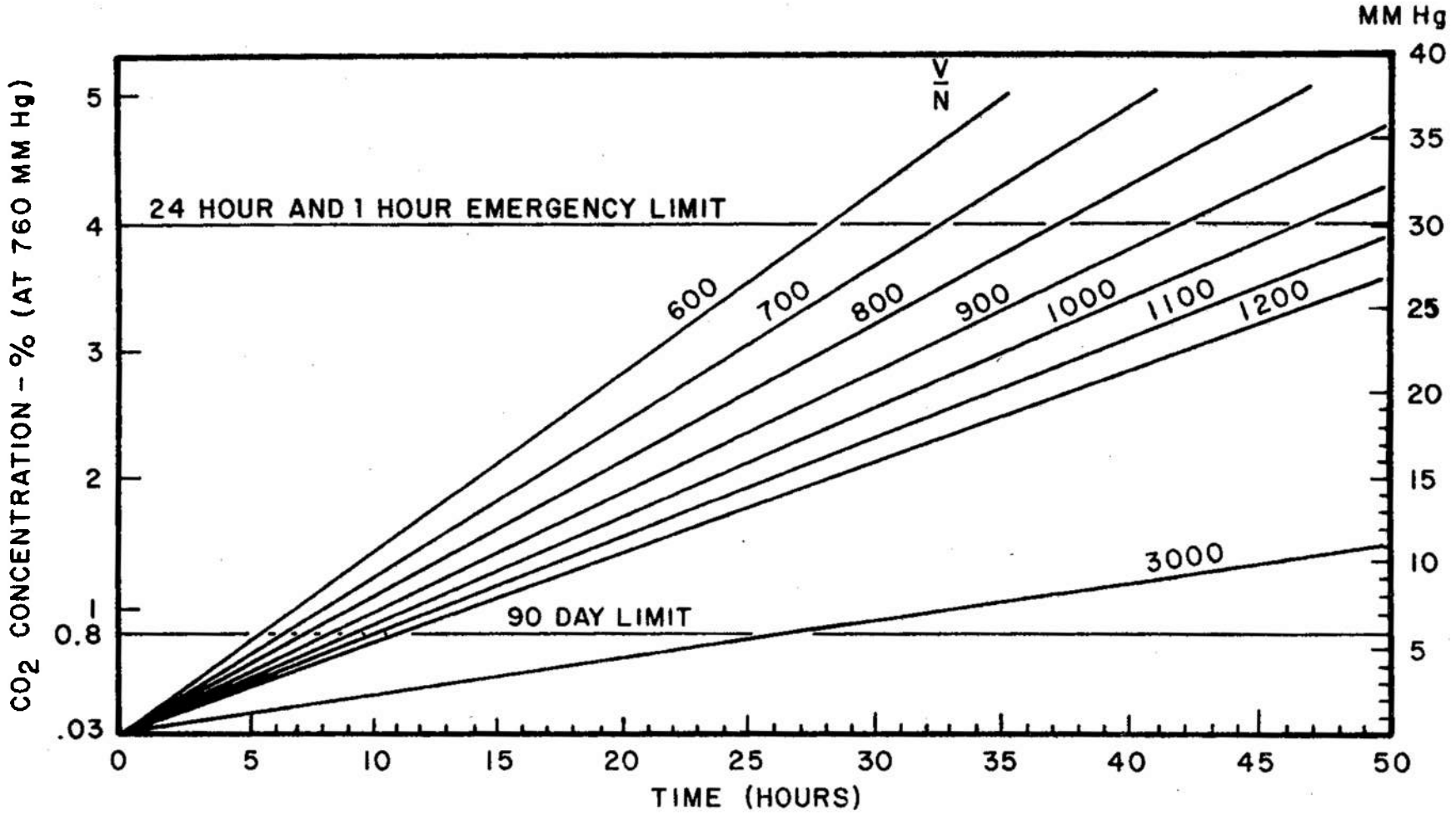

MM Hg
40
35
30
25
20
15
10
5
V/N
24 HOUR AND 1 HOUR EMERGENCY LIMIT
600
700
800
900
1000
1100
1200
3000
90 DAY LIMIT
CO_2 CONCENTRATION - % (AT 760 MM Hg)
5
4
3
2
1
0.8
.03
0
5
10
15
20
25
30
35
40
45
50
TIME (HOURS)

Figure 1

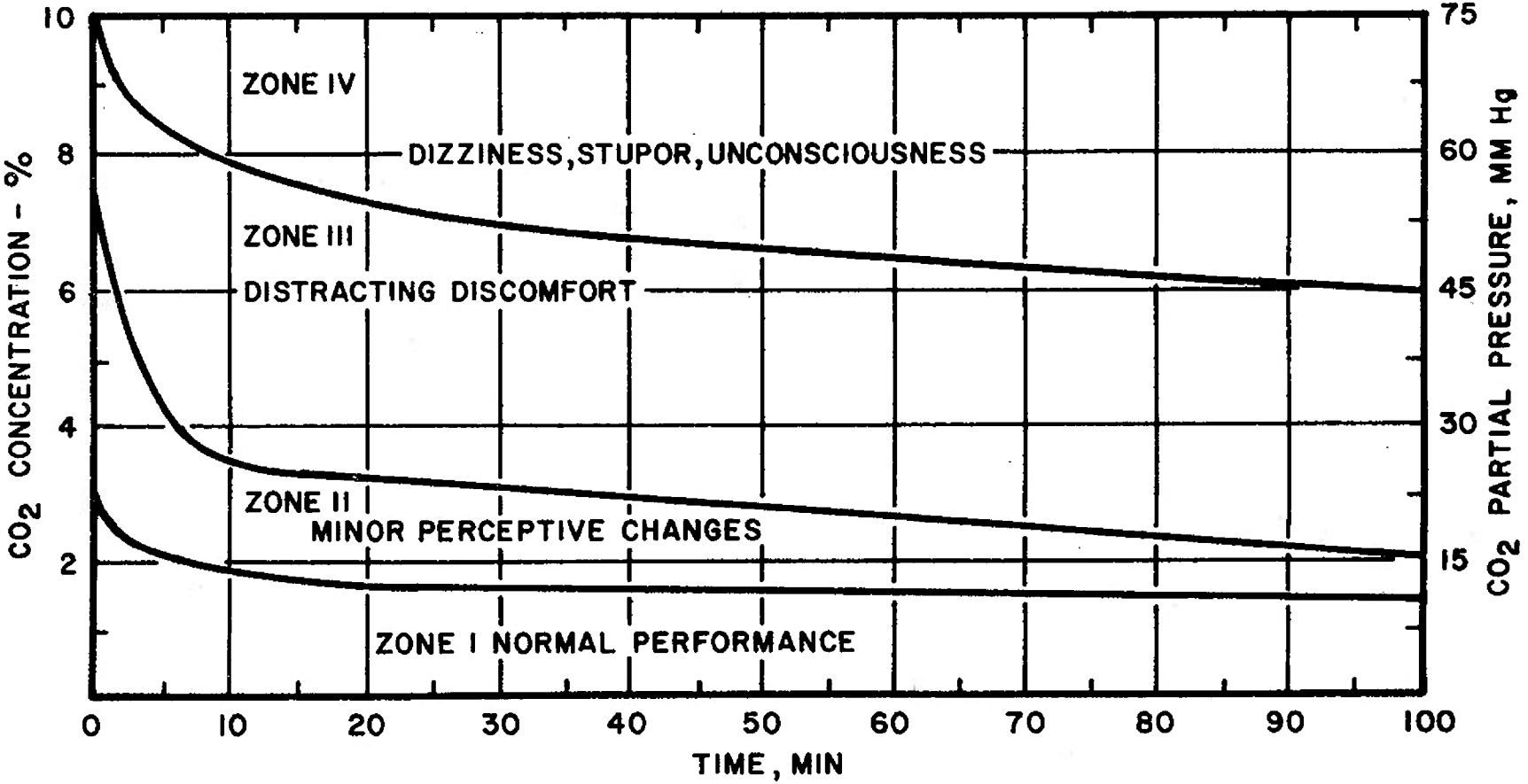

ZONE IV
DIZZINESS, STUPOR, UNCONSCIOUSNESS
ZONE III
DISTRACTING DISCOMFORT
ZONE II
MINOR PERCEPTIVE CHANGES
ZONE I NORMAL PERFORMANCE
CO_2 CONCENTRATION - %
CO_2 PARTIAL PRESSURE, MM Hg
TIME, MIN
10
8
6
4
2
75
60
45
30
15
0
10
20
30
40
50
60
70
80
90
100

Figure 2

of exposure to 3% (21 torr) CO_2 to bring the pH to a level which was still lower than the control value. This was the first evidence of two forms of respiratory adaptation to CO_2, of which only the second one was related to blood bicarbonate changes caused by increased renal reabsorption of bicarbonate. Later, NSMRL conducted a large-scale study, "Operation Hideout", with 21 human subjects exposed to 1.5% (11 torr) CO_2 for 42 days. Significant adaptive changes in respiration, acid-base balance and calcium-phosphorus metabolism were found.

Upon an evaluation of these studies and a review of the literature on chronic CO_2 exposure of humans, Schaefer proposed a "triple tolerance" approach to chronic CO_2 toxicity. His concept described the action of CO_2 at 3 distinct levels. At Level I, 3% (21 torr) CO_2 and above, human performance deteriorated and alterations in basic physiological functions were readily seen. At Level II, 1.5% (11 torr) CO_2, performance was unaffected; however, slow adaptive processes were observed in electrolyte exchange and acid-base balance regulation which were hypothesized to induce patho-physiologic states on greatly prolonged exposure. Level III, at which no significant physiologic adaptive changes to CO_2 occur, has not yet been fully established. At Level I, the renal regulation of bicarbonate reabsorption is fully active. With lower concentrations the renal compensation becomes less effective and, it may be questioned whether or not the kidney is playing an active compensating role. A concentration of 0.5% (3.8 torr) CO_2 may represent a threshold level for the manifestation effects of chronic hypercapnia. It is a level where pathological indicators in animals are slight, physiological functions in humans are not altered significantly, and the CO_2 responses are totally reversible when the subject is returned to breathing normal air (2). NSMRL conducted a workshop on Submarine Medicine in 1978. Formal recognition was given to the minimal health effects of Schaefer's "Level III" exposure to CO_2. Proposals were made for focusing future research on the health consequences of the altered life styles which probably exist during submarine patrols. Such studies might lead to more cost-effective safeguards of health than the high costs of further reduction of CO_2 levels aboard submarines (29, 30).

THE PROBLEM

The length of a nuclear-submarine patrol is currently limited by practical matters such as the food supply and the morale of the crew. Ninety-day patrols are realistic capabilities aboard today's nuclear submarines. The U. S. Navy has adapted an average level of inspired CO_2 of 0.8% (6 torr) as its standard for 90-day exposures to CO_2 and permits maximum concentrations of 4% for brief durations (figure 1 (3)). The rationale for this standard probably evolved from several considerations. Schaefer speculated
(a) that greatly prolonged exposures to 1.5% CO_2 (11.4 torr) could induce pathophysiological changes (in humans): and
(b) that exposures to 0.5 to 0.8% CO_2 (3.8 to 6 torr) probably would not cause significant physiological, or adaptive changes (4).
The limited capabilities of the atmospheric control equipment have imposed considerations of cost-effective compromises between health needs of the crew and space limitations for CO_2 scrubbers aboard submarines (6). Unfortunately there is an apparent lack of scientific and administrative agreement concerning the threshold concentration

of CO_2 at which undesired side effects may occur in humans after prolonged exposures of 30 to 90 days(7).

The practical question is, "Are the inspired levels of CO_2 aboard Naval, nuclear submarines toxic for humans who are continuously exposed for 90 days?" There is no body of scientific knowledge which specifically answers this question. A review of data from the literature was therefore performed in order to comment on the following relevant concerns:

a. Define significant responses to chronic, low-level, CO_2.

b. Assess the dosage of CO_2 necessary for the significant responses during chronic exposures.

c. Ascertain whether or not current levels of CO_2 aboard Naval submarines cause harmful effects in man.

NSMRL has accumulated large sets of data from prolonged animal exposures to CO_2. The findings of the animal work will also be presented as a potential model for possible future studies of chronic, low-level, CO_2 exposures.

HUMAN STUDIES

Literature Review

Low-Level CO_2

A detailed literature review was conducted to assess the responses of humans who have been chronically exposed to low levels of CO_2 (41-65). Some reports of high-level CO_2 exposures were included within the review. A reviewer categorized all of the reported responses into "change" or "no-change". "No change" was judged to be the case by one of the following criteria:

a. The author reported a low-level of statistical significance $p > .05$, by comparison with a control.

b. The reviewer judged, from the variability of the measured response to the CO_2 exposure, that the response would have occurred during a non-CO_2 exposure. Such judgment was required when multiple t-tests were used to test statistical significance rather than the appropriate analysis-of-variance.

"Change" was judged to be the case when:

a. The author reported a high level of statistical significance, $p < 0.05$, by comparison with a control.

b. The reviewer judged that the response resulted from an exposure to CO_2.

General Results of Literature Review

The review yielded 22 separate exposures which embodied data collected from 212 adult men (Table 1). Nine studies were performed in the laboratory and 12 were performed during submarine patrols. The mean P_{ICO_2} was 10 torr (S.D. 7 torr) and the mean duration was 40 days (S.D. 23 days). The ranges of exposure were 3.8 to 30 torr, P_{ICO_2}, during 1 to 90 days of exposure. There were no reports of health impairments attributed to carbon dioxide.

Primary Responses, Pulmonary Function:

The observed rise of the alveolar ventilation in 1 study raised the possibility that low-level CO_2 might stimulate ventilation (Table 2a).

TABLE 1. Literature Review; Conditions of the Exposure

Reference	P_{I,CO_2} Torr	Exposure Time, Days	Number of Men	Laboratory Study	Submarine Patrol Study
A	5	90	4	✓	
B	7.6	63	12		✓
C	30	14	4	✓	
D	11.4	42	23	✓	
E	23	5	5	✓	
F	6.1	20	-		✓
F	6.8	20	10		✓
G	22.8	1	1	✓	
H	3.8	13	6	✓	
I	5.3	52	15		✓
J	3.8	40	9	✓	
J	11.4	12	4	✓	
K	6	64	15		✓
L	6.1	56	10		✓
L	7.6	56	-		
M	7.6	56	31		✓
N	6.5	52	20		✓
O	14	30	6	✓	
P	7.6	40	15		✓
Q	9.1	46	10		✓
R	7.6	54	5		✓
S	?	60	7	—	✓
Mean	10	40			
S.D.	7	23			
Total			212	9	12

TABLE 2a. Summary of Changes, Pulmonary Function

Measurements	"No Change"	"Change"	P_{ICO_2} Mean & S.D.	References
respiratory minute volume	2	5	9.3±3.3	48, 52, 58
tidal volume	2	4	8.6±3.6	48, 52, 58, 61
respiratory frequency	6	1	8.7±3.3	48, 52, 58, 61,62
vital capacity	3		8.4±2.7	48, 58
expiratory reserve volume	1			48
inspiratory reserve volume	1			48
physiological dead space		6	8.1±3.3	48, 52, 58, 61
$\dot{V}_A$		1	11.4	48
P_{ACO_2}		3	9.7±5.3	45, 48, 56, 61
Ventilatory response to CO_2	1	3	9.1±2.7	48, 58

A summary of the pulmonary function changes are given in Table 2a. The respiratory frequency did "not change" while the tidal volume was often found to "change". The physiological dead space "changed" and the respiratory minute volume often changed. The P_{ACO_2} always changed in the few studies reported. There were "no changes" of the vital capacity, inspiratory reserve volume, or expiratory reserve volume. The ventilatory responses to increments of inhaled CO_2 often showed "changes". The data may be presumed to represent changes of the alveolar ventilation by enhancement of the tidal volume.

PRIMARY RESPONSES:
BLOOD GASES AND ACID-BASE:

The arterial P_{CO_2} always "changed" in the few studies reported (Table 2b).

As shown in Table 2b, the mixed venous P_{CO_2} tended to "change" in blood and frozen specimens. The venous pH tended to "change" as did the arterial pH and the capillary pH. The content of bicarbonate tended to "change" in the venous plasma, venous blood, arterial plasma, and arterial blood.

The urinary excretion of ammonium probably "changed" during the exposures. Less dramatic were the "possible changes" of urinary pH, CO_2 content of the urine, and gastric acidity. There was some evidence that "no changes" occurred in the urinary excretion of net acid, titratable acidity of urine, salivary content of CO_2, and urinary excretion of bicarbonate.

Secondary Responses: Electrolytes

The secondary responses to low-level CO_2 were represented by measurements of a wide array of bodily functions (Table 2c). Table 2c summarizes the fluid balance of humans in low level CO_2. The electrolyte metabolism was examined out of concern for possible responses to alterations of the body's acid-base balance. The few studies suggested that the sodium balance could "change". The urinary excretion, plasma concentration, and red blood cell concentration of sodium tended to "change". Although the plasma and erythrocyte concentration of potassium probably "changed", there was the possibility that "no changes" occurred in potassium's balance, urinary excretion, salivary concentration, and fecal excretion. The chloride concentration of the erythrocyte possibly "changed", but neither its plasma concentration nor its urinary excretion seemed altered. There was possibly "no change" of chloride's salivary concentration or fecal excretion. Sparce data suggested that the calcium balance "changed" and that the magnesium balance did "not change". Aside from "no change" of the salivary concentration, and fecal excretion of calcium, the plasma concentration, urinary excretion, erythrocyte concentration, and fecal excretion of calcium tended to "change". "No changes" tended to occur in magnesium's plasma concentration and fecal excretion. The urinary excretion of magnesium and phosphorus probably "changed". The balance of phosphorus tended to "not change". The phosphorus concentration of plasma and excretion in feces tended to "not change", while its salivary concentration tended

TABLE 2b. SUMMARY OF CHANGES, BLOOD GASES AND ACID BASE

Measurements	"No Change"	"Change"	P_{ICO_2} Mean & S.D.	References
P_{aO_2}		2		57,62
P_{aCO_2}, blood or plasma		4	9.8±3.6	50,57,61,62
P_{C,CO_2}	1			56
$P_{\bar{v}}$, CO_2 blood		1	6	57
$P_{\bar{v}}$, CO_2 frozen plasma	1	2	7.6	41,43,59
P_{CO_2}, frozen RBCs		1		43
$[HCO_3^-]$ a, plasma		2	9.5±2.7	50,62
$[HCO_3^-]$ v, blood		3	6.7±0.8	43,57,59
$[HCO_3^-]$ a, blood		1		57
$[HCO_3^-]$ a, RBC		1		50
$[HCO_3^-]$ v, plasma	2	3	8.6±4.0	41,43,49,61
$[HCO_3^-]$ excretion, urine	2	1	7.2±5.89	54,56,61
$[CO_2]$ urine		1	11.4	50
$[CO_2]$ saliva	1		9.1	63
pHc, blood		1		56
pHa, blood		3	9.2±4.2	50,57,61,62
pHa, plasma		1		50,57,61,62
pHv, plasma	2	3	7.6±2.4	41,43,49,59
pH urine	1	1	10.8±4.5	59,60
gastric acidity		1	7.6	64
urinary net acid	2	1	7.7±5.5	56,61
titratable acidity	2	2	9.5±6.4	41,61
24 hour urinary "acid-base balance"	1		5.0	42
urinary ammonium excretion		3	6.7±4.9	55,56,61

TABLE 2c. Summary of Changes, Electrolytes

Measurements	"No Change"	"Change"	References
Na^+ balance	1	1	50,56
Na^+ excretion	1	4	50,56,57,59
$[Na^+]$ plasma	4	4	41,43,44,50,56, 57,59,61
$[Na^+]$ RBC		3	43,50,59
$[Na^+]$ saliva	1		63
K^+ balance	2		50,56
K^+ excretion, urine	4	2	44,50,56,57,59
K^+ excretion, feces	2		56
$[K^+]$ plasma	3	4	41,43,50,57,59,61
$[K^+]$ RBC		1	43,50,59
$[K^+]$ saliva	1		63
Cl^- excretion, urine	3	1	50,56,59,61
Cl^- excretion, feces	1		56
$[Cl^-]$ plasma	4	2	41,43,50,56,57,59
$[Cl^-]$ saliva	1		63
$[Cl^-]$ RBC	1	2	43,50,59
Ca^{2+} balance	1	1	56
Ca^{2+} excretion, urine	2	7	
Ca^{2+} excretion, feces	1	1	56

TABLE 2c. Summary of Changes, Electrolytes, cont.

Measurements	"No Change"	"Change"	References
$[Ca^{2+}]$ plasma	3	5	41,49,55,56,59, 61
$[Ca^{2+}]$ RBC		1	59
$[Ca^{2+}]$ saliva	1		63
Mg^{2+} balance	2		56
Mg^{2+} excretion, urine	1	4	44,55,56,59
Mg^{2+} excretion, feces	2		56
$[Mg^{2+}]$plasma	3	1	55,56,59
Zn^{2+} excretion, urine		1	44
Phosphorus balance	2		56
PO_4^{-3} excretion, urine	4	5	44,49,50,55,56, 57,59
PO_4^{-3} excretion, feces	2		56
$[PO_4^{-3}]$plasma	6	2	41,49,55,56,59, 61
PO_4^{-3} saliva		1	63

to "change". The urinary excretion of zinc possibly "changed".

Secondary Effects: Water Balance

There was strong evidence that the 24-hour urinary volume did "not change" (Table 2d).

There was probably "no change" of the plasma water content or the body weight. Despite possible "changes" of the hematocrit and erythrocyte concentration, sparce evidence suggested "no change" of the water balance, fecal excretion of water, water content of the blood, hemoglobin concentration, flow of saliva, creatinine excretion, and creatinine clearance.

Secondary Effects: Bone

The urinary excretion of hydroxyproline, plasma concentration of calcitonin, and plasma concentration of parathormone tended to "not change". Decrements of vitamin D concentration in plasma have been measured (Table 2e).

Secondary Effects: Metabolism

The metabolism tended to "not change" as evidenced by a few studies showing "no change" of the body temperature, daily intake of food, fecal wet-weight, fecal dry-weight, fecal nitrogen excretion, and nitrogen balance (Table 2f).

Possible "changes" were noted in the respiratory exchange ratio, oxygen uptake, ventilatory excretion of carbon dioxide, and the urinary excretion of nitrogen. Possibilities of "no change" were also found for the plasma concentration of cortisone, the reticulocyte count, and the white blood cell count. Possible "changes" occurred in the polymorphonuclear and eosinophile counts.

Secondary Effects: Cardiovascular and CNS

The blood pressure, heart rate, and psychomotor task performance tended to "no change". Hand steadiness and the electroencephalogram possibly "changed" (Table 2g).

Discussion

A major problem with the conduct of this review was the variety of standards-of-reference for judging significant changes of the measurements. Therefore, the reviewer classified the results into measurements which did or did not change from various reference measurements. The reviewed data collectively represented a continual exposure of men to 1.32% CO_2 (P_{ICO_2}= 10 torr) for 40 days.

A preponderance of the changes were in the functionally related responses of the ventilation, blood gases, and acid-base status of the subjects. Equations predicted that the alveolar ventilation (therefore, the respiratory minute volume) will rise when the inspired levels of CO_2 rise for a given state of metabolic activity (32):

$$\dot{V}_A = \frac{V_{O_2} \cdot k}{P_{A,CO_2} - P_{I,CO_2}}$$

The data from this review showed that the respiratory minute volume changed in response to F_{ICO_2}'s between 0.5 and 2% (3.8 to 15 torr), implicating a more responsive respiratory center than previously thought (17-20).

TABLE 2d. Summary of Changes, Water Balance

Measurements	"No Change"	"Change"	References
water balance	1		56
24-hour urine volume	6	1	55,56,59,61
fecal excretion water	2		56
% water blood	1		49
% H_2O, plasma	2	1	45,49,57
hematocrit	1	2	60,61
erythrocyte count	1	2	45,60,61
[Hb]	1	1	45,60
salivary flow	1		63
creatinine excretion, urine	2		56
creatinine clearance	1		56

TABLE 2e. Summary of Changes, Bone

Measurements	"No Change"	"Change"	References
hydroxyproline excretion, urine	2	1	56, 59
[Vitamin D], plasma		2	56, 65
[Calcitonin] plasma	1		59
[Parathormone] plasma	1		62

TABLE 2f. Summary of Changes, Metabolism

Measurements	"No Change"	"Change"	References
body temperature	1		46
body weight	2	1	46,56
N_2 balance	1		50
daily food intake	1		56
fecal N_2 excretion	1		50
fecal wet-weight	1		56
fecal dry-weight	1		56
N_2 excretion, urine		1	50
respiratory exchange ratio	1	1	50,61
$\dot{V}_{O_2}$	1	1	48,61
$\dot{V}_{CO_2}$	1	1	48,61
ventilatory excretion CO_2		1	45
[Cortisol] plasma	1		62
Reticulocyte count	2		45,60
white blood cell count	1		45
[EOS's]		1	45
[PMN's]		1	45

TABLE 2g. Summary of Changes, Cardiovascular and CNS

Measurements	"No Change"	"Change"	References
test of performance	2		45,61
blood pressure	1		46
heart rate	1		46
hand steadiness		1	51
EEG		1	51
EEG, sleep		1	45

The stability of the body water balance, nitrogen balance, and body temperature were strongly indicative that chronic, low-level CO_2 (10 torr for 40 days) did not disturb the basic metabolic needs of the body.

Dose Response Trends in Chronic Exposures to Low-Level CO_2

Attempts were made to determine the dosage of CO_2 at which definable responses could be measured (Figures 3 and 4). One type of CO_2-dosage studied was the P_{ICO2} of the ambient environment in which the men resided for days at a time (Figure 3). Using the "change" and "no change" data from the Literature Review, the alveolar and arterial P_{CO2}'s were found to "change" at P_{ICO2} 3.8 torr (Figure 3). The ventilatory responses appeared to be very sensitive to inspired CO_2 levels $\geq$ 6 torr. The venous plasma pH changes were variable, but the arterial pH's "changed" at $P_{ICO2} \geq 6$ torr (figure 3b). The venous bicarbonate content "changed" at $P_{ICO2} > 6$ torr. There was a "change" of the urinary ammonium excretion above CO_2 levels of 3.8 torr but not necessarily a "change" of the urinary net acid excretion. The responses of the inorganic ions to low-level CO_2 were quite variable (figure 3c). The rates of urinary excretion of calcium and phosphorus "changed" over a wide domain of levels of CO_2 (5 to 30 torr). However, there were occasions when "no change" of the excretion of calcium and phosphorus occurred during exposures to $P_{ICO2} < 12$ torr. The plasma concentrations of calcium, sodium, and potassium showed both "changes" and "no changes" at CO_2 levels between 3 and 15 torr. In summary, acid-base balance and the blood gases were very sensitive to low levels of P_{ICO2}. The body fluid and electrolyte metabolism responded with great variability and showed no clear trend toward a threshold effect.

CO_2 doses were also examined by expressing them as a combined effect of CO_2-level and duration-of-exposure. The product of P_{ICO2} and days-of-exposure (torr-days) was computed for those exposures whose responses were just discussed (figure 4). "Changes" occurred in the respiratory minute volume, P_{ACO2}, and $P_{a,CO2}$ when the CO_2 dose $\geq$ 150 torr-days (figure 4a). CO_2 doses $\geq$ 300 torr-days were accompanied by "changes" of the arterial pH, whereas the venous bicarbonate content and venous pH contained a mixture of "change" and "no change" with increased CO_2 dose (figure 4b). The urinary ammonium excretion "changed" and the urinary net-acid excretion did not necessarily change at CO_2 doses $\geq$150 torr-days (figure 5b). No dose-response trends were apparent for the response of the inorganic ions between 125 and 500 torr-days (figure 4c).

The nature of the dose-response trends from chronic, low level CO_2 exposures suggests that the ventilatory and acid-base functions "changed" above a threshold dosage. The respiratory minute volumes transitioned from "no change" to "change" as the CO_2 doses rose above 7 torr and 300 torr-days (figure 3a,4a). The arterial and alveolar P_{CO2}'s always showed "change" down to 3.8 torr and 150 torr-days (figure 3a, 4a); and there was no transition of $P_{A,CO2}$ or $P_{a,CO2}$ into a "no change" status at low doses of CO_2. There was no transition of the arterial pH into a "no change" status below the doses which showed "change", namely $\geq$ 6 torr and $\geq$ 300 torr-days (figures 3b and 4b). The notable feature of the responses of the

Fig. 3 a

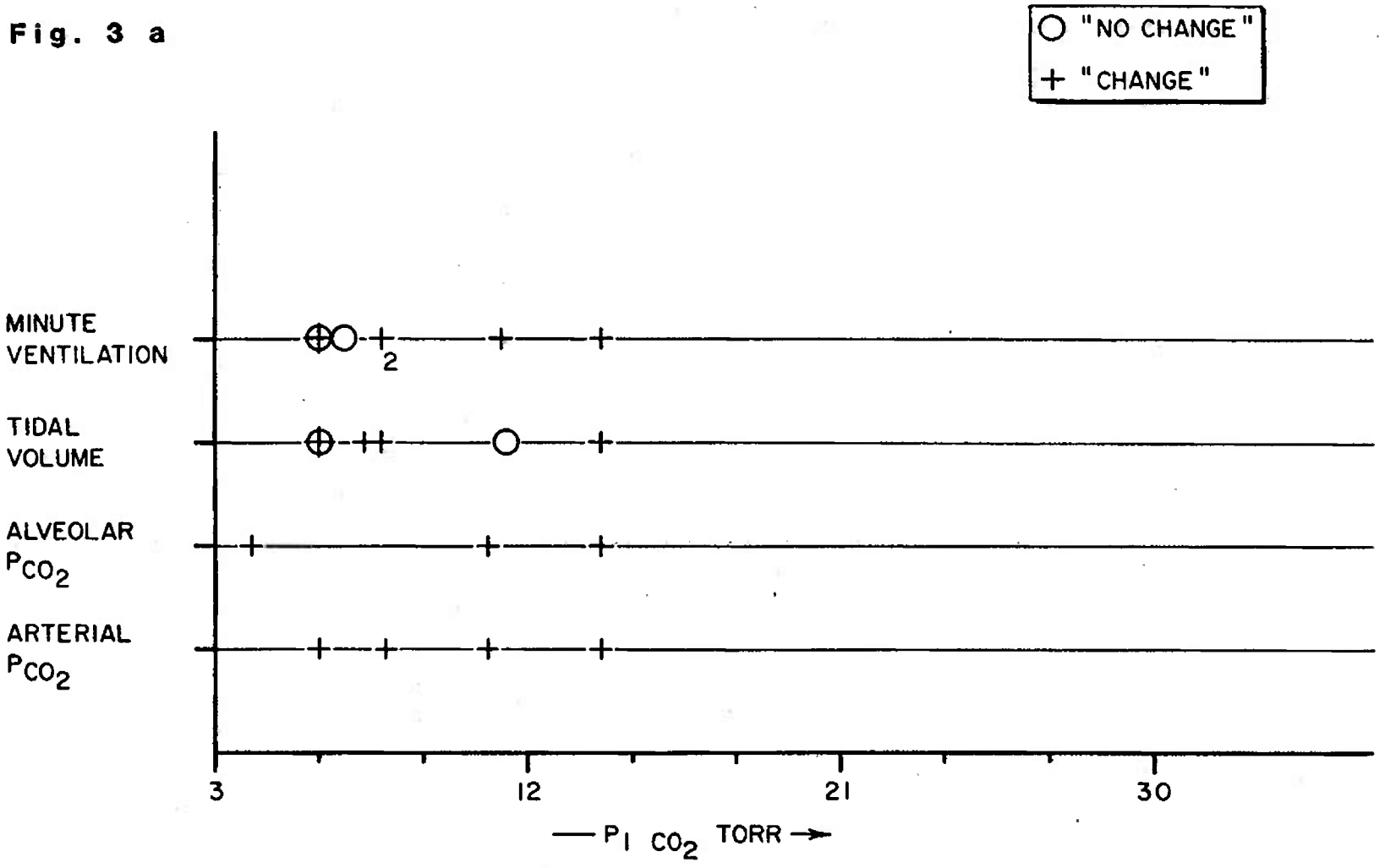

"NO CHANGE"
"CHANGE"
MINUTE VENTILATION
TIDAL VOLUME
ALVEOLAR P_{CO_2}
ARTERIAL P_{CO_2}
2
3
12
21
30
$P_I\ CO_2$ TORR

Fig. 3 b

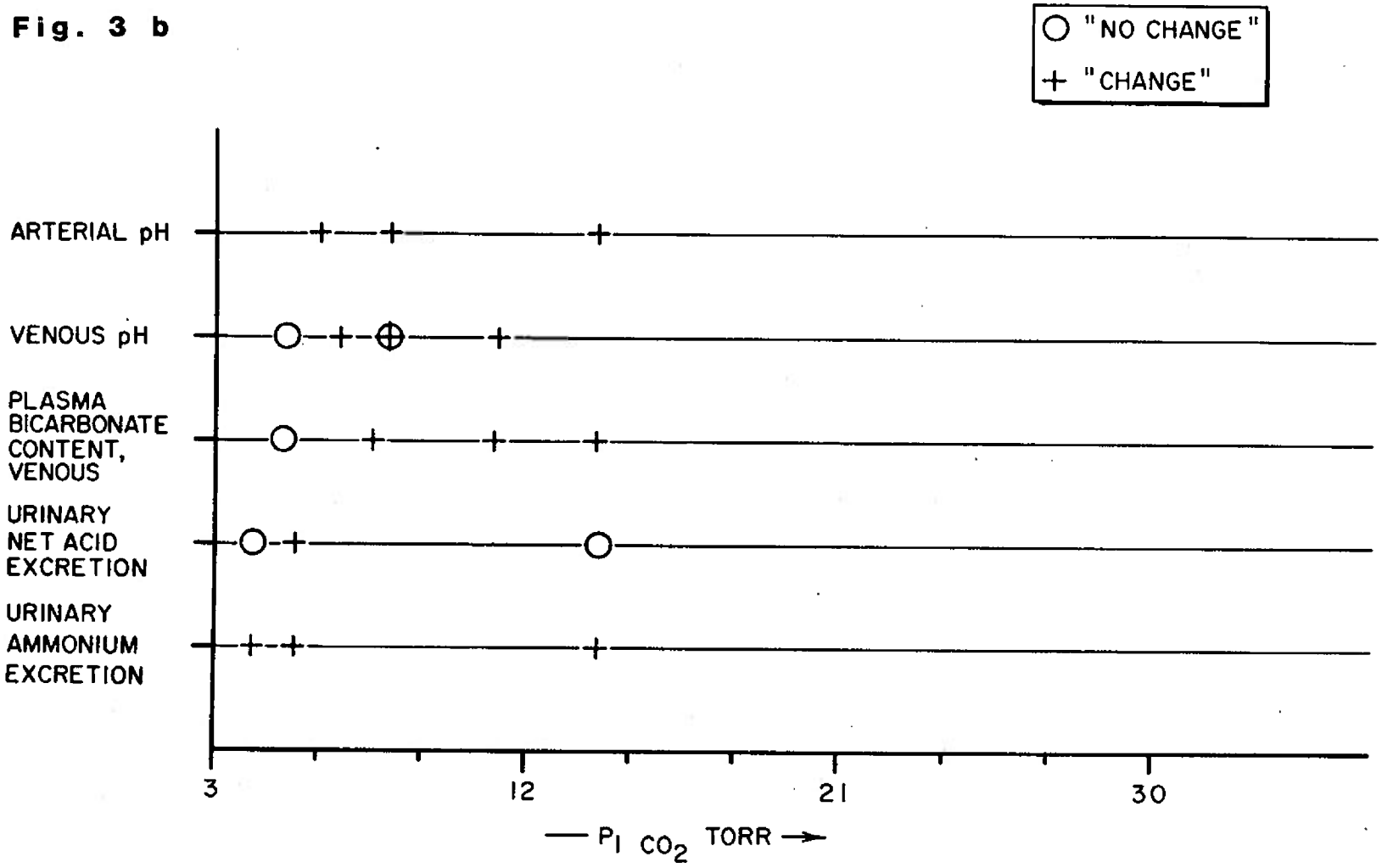

○ "NO CHANGE"
+ "CHANGE"
ARTERIAL pH
VENOUS pH
PLASMA BICARBONATE CONTENT, VENOUS
URINARY NET ACID EXCRETION
URINARY AMMONIUM EXCRETION
3
12
21
30
— P_I CO_2 TORR →

Fig. 3 c

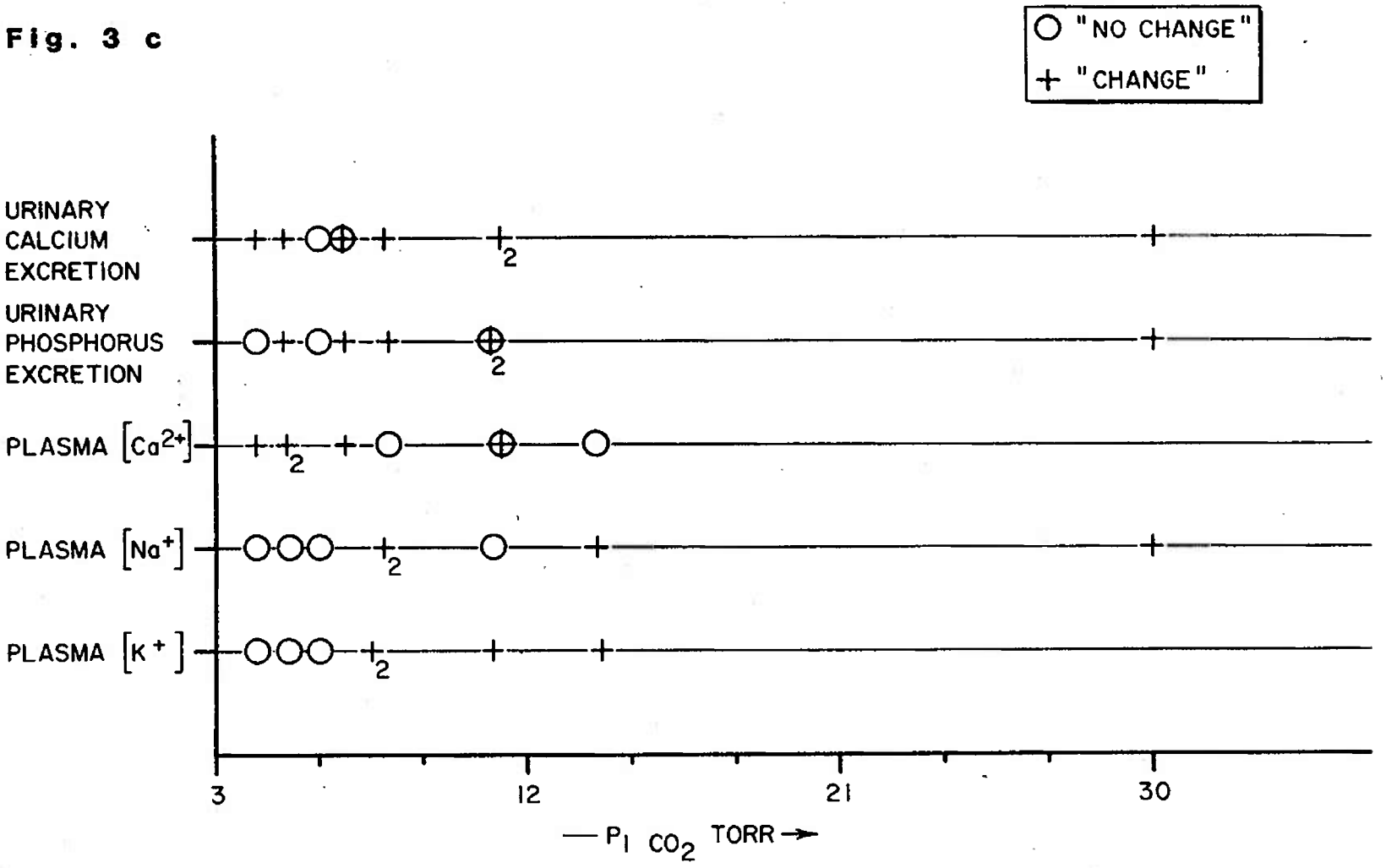

"NO CHANGE"
"CHANGE"
URINARY CALCIUM EXCRETION
URINARY PHOSPHORUS EXCRETION
PLASMA [Ca2+]
PLASMA [Na+]
PLASMA [K+]
3
12
21
30
— PI CO2 TORR →

Fig. 4 a

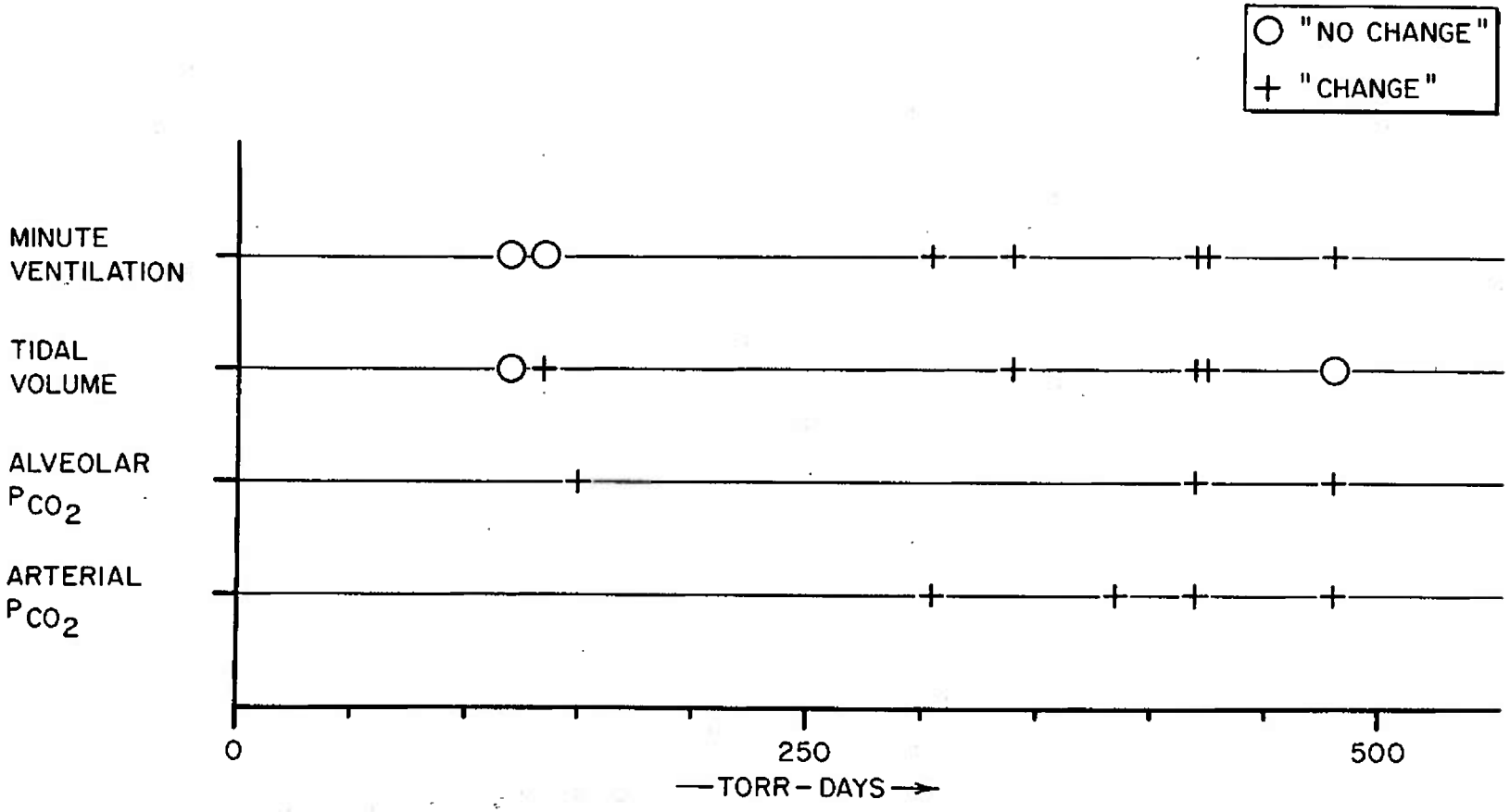

"NO CHANGE"
"CHANGE"
MINUTE VENTILATION
TIDAL VOLUME
ALVEOLAR P_{CO_2}
ARTERIAL P_{CO_2}
0
250
500
—TORR – DAYS→

Fig. 4 b

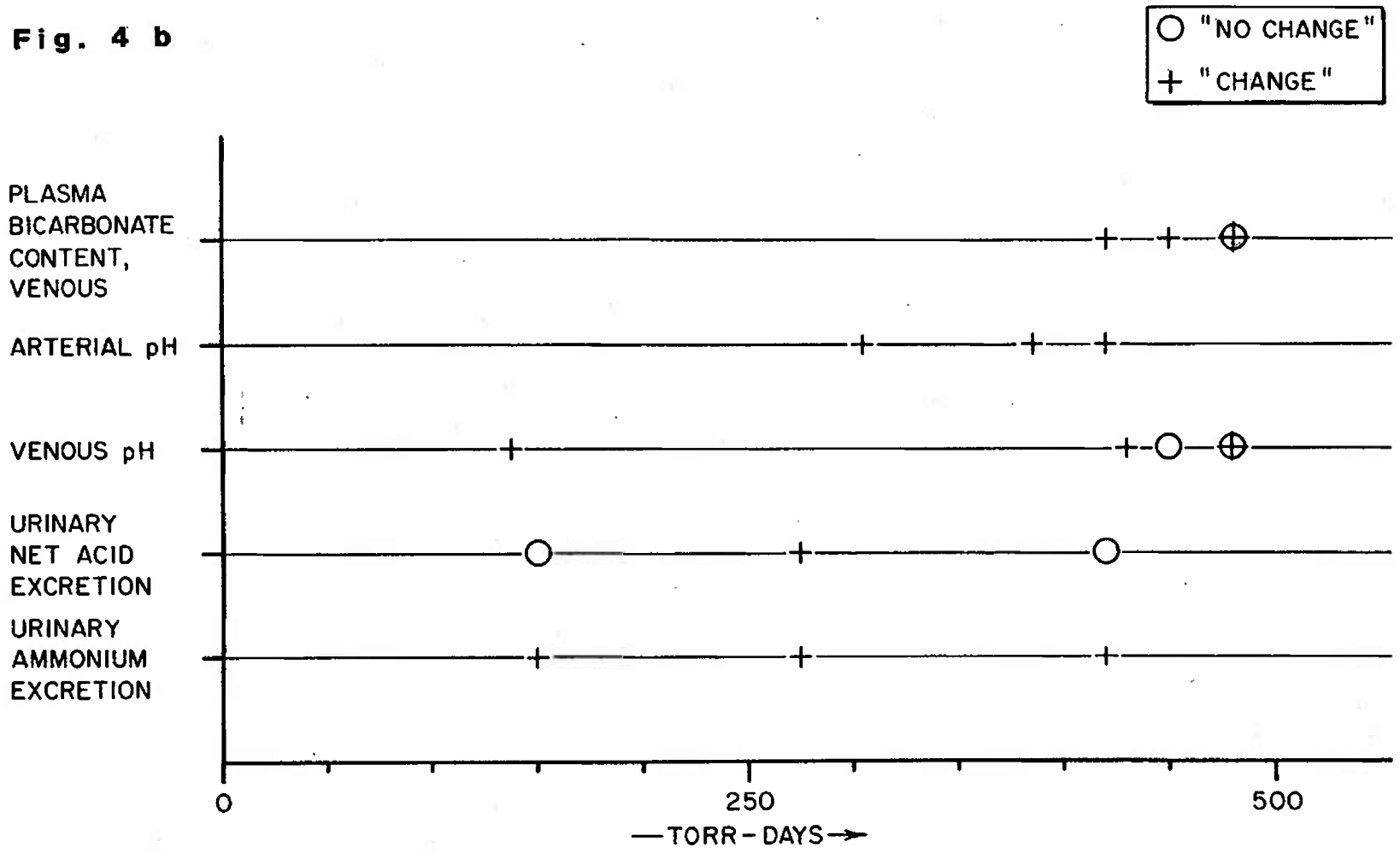
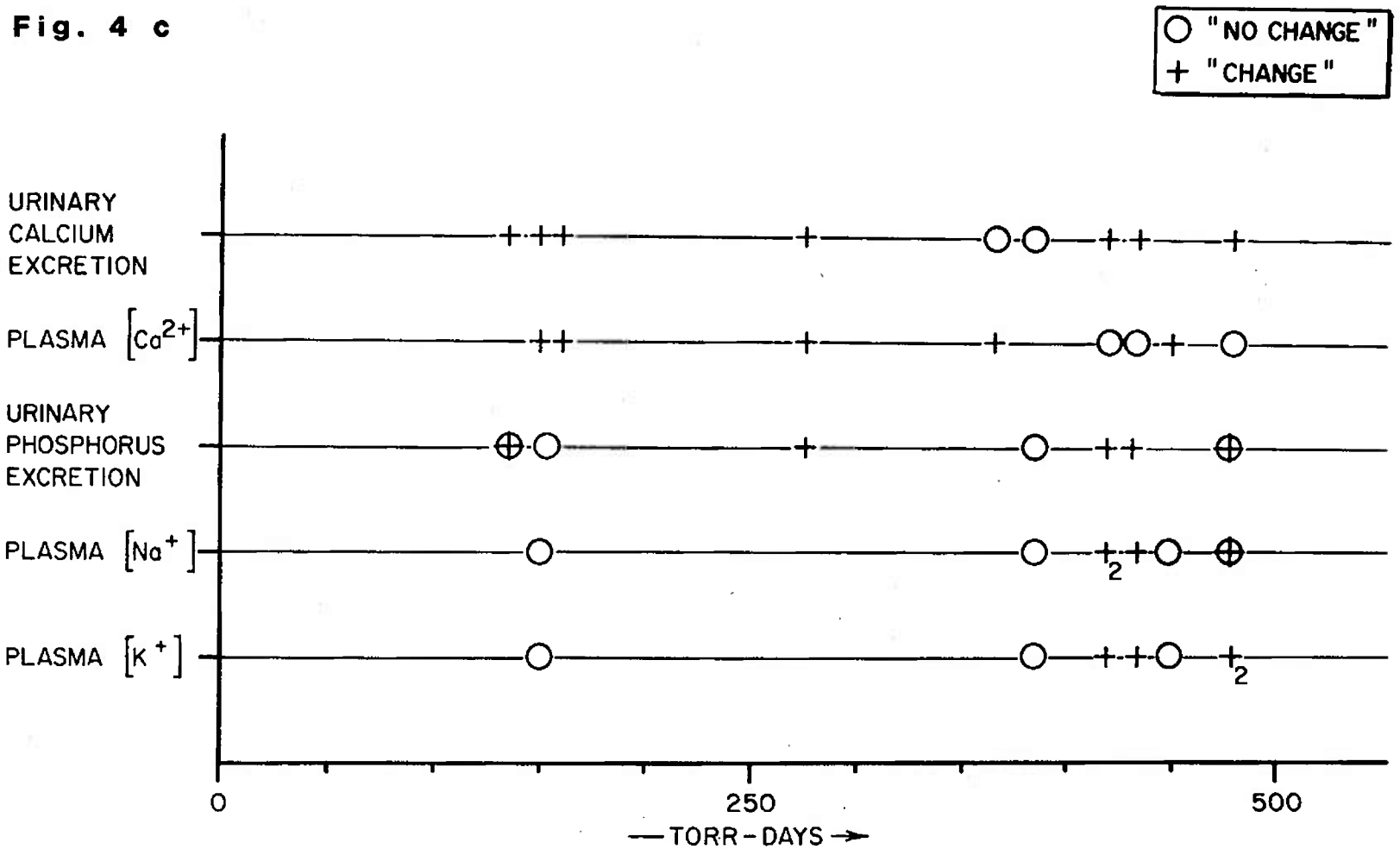

"NO CHANGE"
"CHANGE"
PLASMA BICARBONATE CONTENT, VENOUS
ARTERIAL pH
VENOUS pH
URINARY NET ACID EXCRETION
URINARY AMMONIUM EXCRETION
0
250
500
—TORR-DAYS→

Fig. 4 c


"NO CHANGE"
"CHANGE"
URINARY CALCIUM EXCRETION
PLASMA [Ca2+]
URINARY PHOSPHORUS EXCRETION
PLASMA [Na+]
PLASMA [K+]
0
250
500
— TORR - DAYS →

inorganic ions to graded doses of CO_2 was the preponderant lack of a clear transition from "no change" to "change" (figures 3c,4c).

Thus, the threshold dosage of CO_2 below which many physiological functions do not "change" appears at $P_{ICO_2} < 6$ torr; and at P_{ICO_2} X time < 150 torr-days (figures 3,4). Another way to view the primary and secondary responses to doses of chronic, low-level CO_2 is to perceive a continuum of functional responses as the doses become lower. At some point, the secondary responses may occur despite an absence of the primary responses. The reason for this is uncertain at this time.

Safe 90-Day Exposures

Laboratory Studies: 3.8-5 Torr

The 90-day and 40-day human exposures to 3.8-5 torr resulted in no change of the pulmonary ventilation (41,42,56). The arterialized capillary pH fell by only 0.02 pH-units, which is generally agreed to be a small, clinically insignificant change. The alveolar and mixed venous P_{CO_2}'s rose by 1-1.5 torr whereas the arterialized capillary P_{CO_2} rose 4 torr. The net-acid excretion by the urine was unchanged, but other urinary excretion rates of bicarbonate and ammonium ions rapidly rose to sustained levels during the CO_2 exposure. The subjects developed a positive sodium balance with a decline of their urinary excretion of Na^+. Their plasma concentrations of Na^+, water balance, magnesium balance, phosphorus balance, and potassium balance did not change. The body weights and 24-hour urinary volumes did not change. The calcium balance was transiently negative, the plasma levels and fecal excretion of calcium were increased, and the urinary excretion rate of hydroxyproline did not rise. The plasma vitamin D levels were decreased (41,42,56).

90 Day Exposures of Primates

At least 2 studies have been conducted in which the responses of primates were measured during continuous exposures to CO_2 for 90 days. Monkeys were exposed to 3% CO_2 and 21% O_2 for 93 days. The CO_2-exposed monkeys fared as well as controls who were similarly confined in air. The CO_2 exposed monkeys showed no significant changes of their body weight, activity, hematocrit, blood glucose, total leukocyte count, serum chloride, serum calcium, serum phosphorus, erythrocyte sedimentation rate, or serum bilirubin. Autopsied animals showed only the presence of mites in the lung. There was no evidence of adrenal impairment (5). Humans were exposed to 1% CO_2 at 0.7 ATA (P_{ICO_2} = 5 torr) for 90 days. Analyses of frozen, anaerobic venous plasma samples suggested the existence of a mild respiratory acidosis during the middle and latter phases of the exposure to CO_2. The data from the CO_2-exposed subjects were statistically analyzed with respect to their values measured during the recovery from the exposure. Aside from a case of Streptococcal infection, the subjects did not develop any significant medical problems during the CO_2 exposure (41,42). The combined information literally suggests that humans may be able to safely tolerate living in 5 torr CO_2 for 90 days without endangerment to their health. Non-injurious phases of respiratory acidosis may be expected to occur (5,26,41,42).

NSMRL ANIMAL CO_2 EXPOSURES

Studies at this laboratory have shown that different animal species exhibit various responses to CO_2. To better understand this phenomenon, guinea pigs and rats were exposed to different carbon dioxide (CO_2) gas mixtures for periods of one hour, one day, and seven days (39). Carbon dioxide titration, absorption, and buffer curves were determined for both species in order to interpret the buffer capacity of the organism as a result of a sudden increase in respired CO_2. Compared to rats, guinea pigs were found to be less tolerant to a given CO_2 load during the initial stages of a CO_2 exposure. As early as one day of an exposure to CO_2, rat blood was better buffered than guinea pig blood. By the seventh day of the exposure both species exhibited similar responses indicating that the guinea pigs had developed a metabolic adjustment to the increased CO_2 load. An explanation for this species difference may be related to the fact that in the guinea pig's kidney the two enzymes glutaminase I and phosphoenolpyruvate carboxykinase occur at a much lesser concentration than in the rat. These enzymes are known to be a factor in the metabolic response of the kidney when the acid-base balance of the organism is altered and in the guinea pigs may result in decreased buffering of an increased amount of CO_2 in the blood. Other factors such as differences in body temperature and an alternate reabsorption of body minerals by the kidney of the guinea pig may also be involved.

Lung changes have also resulted from exposure to CO_2. Douglas, et al reported that 1.0% CO_2 exposure for 4 and 6 weeks produced ultrastructural changes in guinea pig lungs (36). Significant increases in Type II pneumocyte cell size were observed, as well as increases in the number and size of lamellar bodies in these cells. Recovery was incomplete after 4 weeks. Schaefer, et al demonstrated an increase in kidney calcium content of guinea pigs after only 2 weeks exposure to 1.0% CO_2, persisting until 6 weeks (37). No recovery studies were done in this experiment. There was a persistently elevated plasma calcium in these animals which became significantly elevated only after 6 weeks. Experiments were then carried out at 0.5% in which guinea pigs exposed for 8 weeks showed no significant lung structure changes. There was, however, a significant increase in kidney calcification after 8 weeks, which returned to normal levels after 4 weeks recovery on air. Plasma calcium was reported to be significantly elevated at 8 weeks (38).

To determine a threshold level that elicits kidney and lung alterations, Shea, et al exposed a large number of guinea pigs under the same experimental conditions while varying only the CO_2 concentration (0.1, 0.2, 0.3, and 0.5%) and the time of exposure (3,6,9,and 13 weeks)(22). The results of these experiments are as follows: After 6 weeks exposure to 0.1%, 0.2%, 0.3% or 0.5% CO_2, there was no significant change in cell diameter, lamellar bodies (LB) per cell, or LB diameter (Table 3). However, after a 9-week exposure there was an increase in Type II cell diameter in guinea pig lung exposed to 0.3% CO_2 and to a greater extent those exposed to 0.5% CO_2; no changes in LB numbers per cell or LB size were observed. At 13 weeks exposure, cells in 0.2% CO_2 showed a slight increase in lamellar body number. Type II cells exposed to 0.3% and to a much greater extent, 0.5% displayed a marked increase in all

TABLE 3. Changes in Type II Pneumocytes of Guinea Pigs Exposed to a Range of CO_2 Concentrations

	Cell Diameter (μm)	Lamellar Bodies per Cell (No.)	Lamellar Body Diameter (μm)
(1) 6 Week Controls	9.0±1.5	5.0±1.6	1.0±0.08
6 Week 0.1% CO_2	9.0±1.4	5.1±1.4	1.2±0.07
6 Week 0.2% CO_2	9.1±1.3	5.0±1.3	1.3±0.05
6 Week 0.3% CO_2	9.1±1.8	5.2±1.1	1.0±0.08
6 Week 0.5% CO_2	9.2±1.6	5.1±0.9	1.1±0.06
(2) 9 Week Controls	9.1±1.2	5.1±1.3	0.9±0.05
9 Week 0.1% CO_2	9.1±1.1	5.1±1.4	1.1±0.06
9 Week 0.2% CO_2	9.3±1.08	5.2±1.3	1.2±0.07
9 Week 0.3% CO_2	9.8±0.93	5.2±1.2	1.1±0.05
9 Week 0.5% CO_2	11.2±1.2	5.1±1.1	1.1±0.08
(3) 13 Week Controls	9.2±1.4	5.3±1.0	1.3±0.06
13 Week 0.1% CO_2	9.0±1.3	5.4±1.5	1.4±0.01
13 Week 0.2% CO_2	9.3±1.9	5.6±1.1	1.5±0.03
13 Week 0.3% CO_2	10.5±1.1	7.5±1.2	2.1±0.06
13 Week 0.5% CO_2	15.3±1.9*	9.8±1.9*	3.1±0.07*
(4) 13 Week Controls, Plus 4 Week Recovery	9.3±1.2	5.2±1.2	1.2±0.08
13 Week 0.1% CO_2, Plus 4 Week Recovery	9.2±1.1	5.1±0.08	1.2±0.07
13 Week 0.2% CO_2, Plus 4 Week Recovery	9.4±1.2	5.2±1.0	1.1±0.03
13 Week 0.3% CO_2, Plus 4 Week Recovery	9.2±1.4	5.1±1.1	1.3±0.03
13 Week 0.5% CO_2, Plus 4 Week Recovery	10.5±1.4	5.1±1.3	1.5±0.05
(5) 13 Week Controls, Plus 8 Week Recovery	9.2±1.6	5.1±1.2	1.1±0.05
13 Week 0.1% CO_2, Plus 8 Week Recovery	9.6±1.2	5.2±1.3	1.2±0.01
13 Week 0.2% CO_2, Plus 8 Week Recovery	9.7±1.1	5.4±1.0	1.4±0.02

TABLE 3. Changes in Type II Pneumocytes of Guinea Pigs Exposed to a Range of CO_2 Concentrations, cont.

	Cell Diameter (μm)	Lamellar Bodies per Cell (No.)	Lamellar Body Diameter (μm)
13 Week 0.3% CO_2, Plus 8 Week Recovery	9.3±1.3	5.1±0.08	1.5±0.03
13 Week 0.5% CO_2, Plus 8 Week Recovery	9.5±1.0	5.0±0.09	1.1±0.02

The mean cell diameters of Type II alveolar epithelial cells are measured in micrometers (μm). The mean number of lamellar bodies per Type II alveolar epithelial cell was also calculated. The mean lamellar body diameters are measured in micrometers (μm).

* Statistically Significant

three parameters relative to control values. After exposure for 13 weeks to CO_2 followed by a 4-week recovery period in room air the Type II cells obtained from lungs of animals exposed to 0.1, 0.2, and 0.3% CO_2 remained at, or returned to control values. Type II cells exposed to 0.5% CO_2 for 13 weeks allowed to recover for 4 weeks still retained an increased cellular diameter compared to control values; the number of LB per cell and size of LB, however, did return to control values. These results suggest that the Type II cell is sensitive to CO_2 concentration and that there is a hypertrophic response of the cell cytoplasm after 9 weeks exposure to 0.5% CO_2. Following 13 weeks exposure to 0.3 and 0.5% CO_2 the numbers of LB per cell and LB diameter also increased, suggesting that the synthesis of pulmonary surfactant is stimulated after prolonged exposure to 0.3 - 0.5% CO_2. This change in the Type II cell is not permanent. If the animal is allowed to recover in room air for 4 weeks, LB values return to control values and the hypertrophic response of the Type II cell cytoplasm also returns toward control values form a significantly elevated state. After 8 weeks of recovery following 13 weeks of exposure to 0.5% CO_2, all lung parameters measured returned to control values. In summary, the Type II cell of the guinea pig responds to increased CO_2 levels first by cellular hypertrophy and later by increased synthesis of pulmonary surfactant (evidenced by increased numbers and size of LB). These parameters return toward control values after recovery in room air (22).

In the same experiment by Shea, et al a significant increase in kidney calcium was obtained for 6, 9, and 13 weeks of 0.5% CO_2, and for 9 and 13 weeks at 0.3% (Figure 5). As in the lung tissue, kidney calcium values return back to normal when the animals are allowed to recover on air after a 13-week exposure.

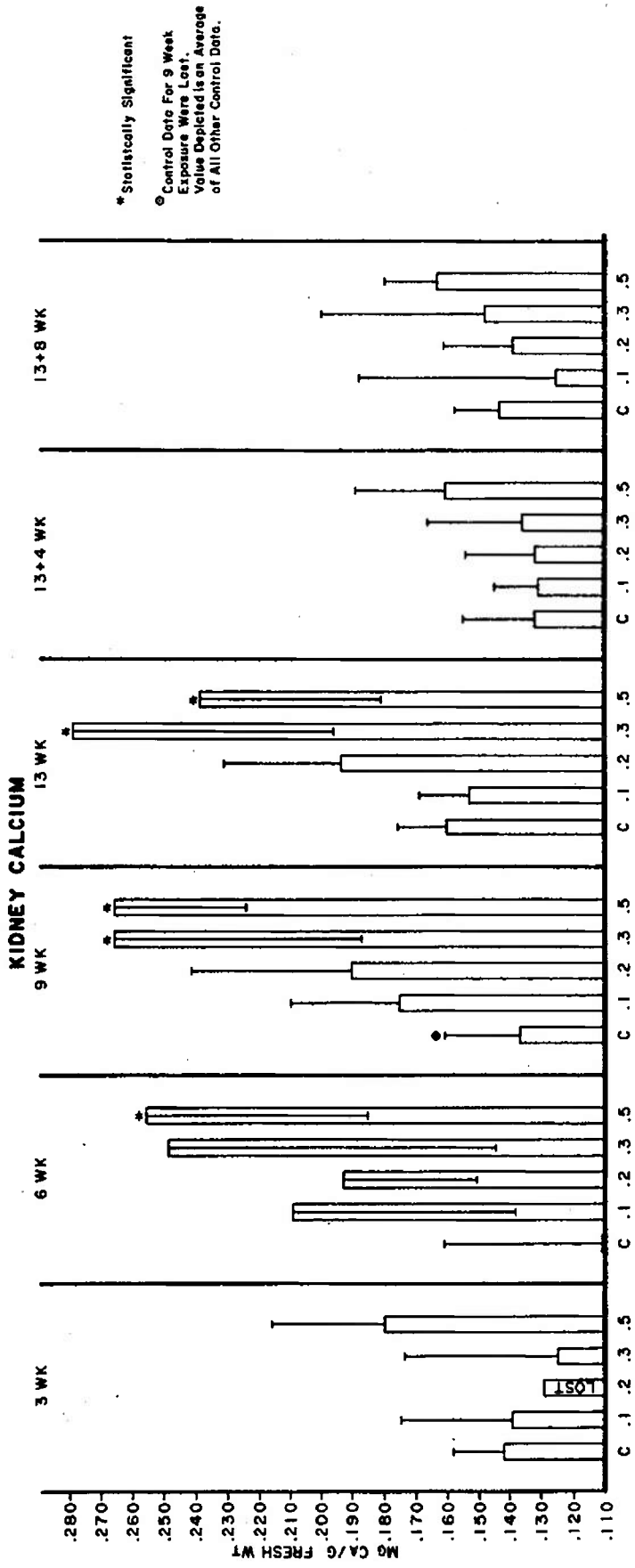

*Statistically Significant
©Control Data For 9 Week Exposure Were Lost. Value Depicted is an Average of All Other Control Data.
KIDNEY CALCIUM
MG CA/G FRESH WT
3 WK
6 WK
9 WK
13 WK
13+4 WK
13+8 WK
LOST
C .1 .2 .3 .5

Figure 5

In addition to the above parameters, blood gases, ionized plasma calcium, total plasma calcium, bone, heart, muscle, and lung calcium and tissue water content were analyzed. These parameters, as well as intracellular pH, show no significant changes from control for any exposure. It is surprising that the plasma calcium did not show positive results since it did in previously published 0.5% CO_2 experiments (22).

Thus in a sensitive animal model, there appears to be a definite physiologically indicated threshold to CO_2 exposure and most importantly, alterations in structure seem to be fully reversible upon return to ambient air.

CHRONIC LOW-LEVEL CO_2 TOXICITY?

The U. S. Navy continues to search for more feasible methods of further reducing the levels of CO_2 aboard its nuclear submarines. Such an expensive practice stems from a concern for toxic effects from prolonged exposures to low levels of CO_2. These toxic effects, if they exist, have not been defined! Reasoning suggests that CO_2 could become toxic when the body can no longer maintain tolerable levels of CO_2 content. One of the following effects theoretically represents a toxic response of humans to chronic, low-level CO_2:

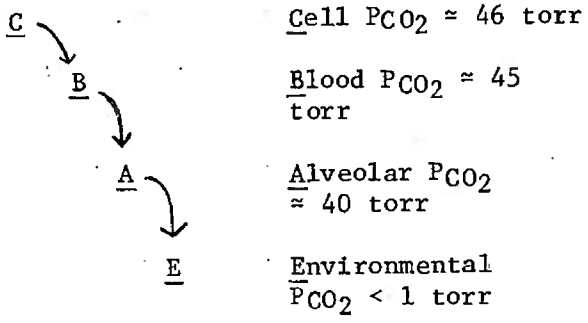
a. Cellular injury.
b. A lowering of the human's defenses against stress.
c. A reduction of human performance.
d. A change of human behavior.
e. A shortening of life expectancy
f. Oncogenesis or genetic mutations.

However, a universal finding of all the articles and data reviewed in this manuscript was the lack of documented toxic effects from the exposures (41-65). The lack of incriminating evidence would permit a reasonable presumption that chronic, low-level CO_2 exposures are non-toxic!

The body's responses to inhalation of low and moderate concentrations of CO_2 may be physiological and reversible rather than toxic and damaging. This view was shared by Lambertsen, who suggested that lifetime exposures to $\leq$ 1% CO_2 could be tolerated without detectable limitations (26). The extent to which the atmospheric conditions aboard submarines affect the health of the crewmembers appears to be minimal (25,29). According to a recent review of the health effects of the nuclear submarine atmosphere, it was found that the levels of CO_2 in Naval submarines dropped below 1% after 1967. Headaches, eye-ear-nose-throat symptoms, injuries, and infections could not be attributed to atmospheric causes in either the " $\geq$ 1% $FICO_2$" or the " < 1% $FICO_2$" submarine environments (25).

The long-term effect of the submarine atmosphere on submariners' health is currently being investigated by NSMRL and the Department of Epidemiology at Yale University. Morbidity and mortality data from active duty and retired submariners will allow the most critical assessment to date of the health effects of exposure to Naval nuclear submarines (27). The results of the study may allow an estimation of the effect of the total environment aboard submarines on the life expectancy and stress tolerance of submariners.

Perhaps the proper measurements of chronic, low-level CO_2 toxicity were not measured in the studies reviewed. It is not too difficult to postulate a mechanism for the development of chronic, low-level CO_2 toxicity. Consider, briefly, the analogy of hyperthermia. Sudden exposures of humans to heat stress cause remarkable physiological adjustments to occur. These adjustments may prevent the rise of body temperature, and therefore the net gain of body heat. On the other hand, the condition of hyperthermia is always accompanied by a gain of the body's heat content and a rise of the core temperature. From this analogy, it is suggested that the sine qua non for any form of CO_2 toxicity is the forced expansion of the tissue content of CO_2. The tissue CO_2 content is controlled by a cascade of P_{CO_2}'s which drive the transport of CO_2 from the cells to the environment;



The cellular CO_2 diffuses quite rapidly into the blood. The net transport of CO_2 out of the tissue is limited by the tissue blood flow; therefore, the cellular P_{CO_2} would rise if the customary tissue blood flow were to be reduced. After entering the venous blood, the CO_2 from the mixed-venous blood diffuses rapidly into the alveoli (8,9). An important implication of the P_{CO_2} cascade is that elevation of the environmental load of CO_2 results in elevation of the blood and cellular P_{CO_2} (21).

The experimental determination of those chronic exposures to low-level CO_2 which are toxic to humans rests on a basic assumption that there are safe tolerance limits for the net gains or losses from the body's CO_2 stores. Safe limits for acute changes of the body content of CO_2 have been suggested. Tetany may develop upon the loss of 3.5 liters of CO_2 per m^2 body surface area. Narcosis can develop in a 70-kilogram man when he gains 3 liters of CO_2 at a P_{CO_2} of 70 torr. The range of soft-tissue stores of CO_2 permitting accustomed performance in men was estimated to be 14 to 23 liters (11,12). Safe limits for chronic changes of the body content of CO_2 have not been suggested. The tissue stores of CO_2 have apparently not been studied in men exposed to chronic, low level CO_2. The largest soft tissue storage site is muscle, while bone represents an even larger repository for CO_2 (8,11,12). Animal studies have shown that bone is capable of accumulating larger quantities of CO_2 with multi-week exposures to 0.3 to 1.0% CO_2. Of particular interest was the increased content of calcium within the kidney and decreased content of calcium in the bone (22, 28). Animals are not humans, however, so that it remains to show whether or not the hard or soft tissues of humans store CO_2 during residence in such comparably low levels of CO_2. Recent evidence suggested that submarine patrols do not alter the calcium content of bones in submariners (23). If submariners experience increases of their bone CO_2 stores they may do so without mobilizing calcium from their bone.

The difficulties with documenting chronic, low-level CO_2 toxicity in the laboratory are the tremendous costs of performing such studies and the insensitivity of the laboratory

measurements. There is a certain amount of "noise" inherent within the various biophysical measurements of CO_2 toxicity. For example, there is an error in accuracy of 1-2% with usage of the P_{CO_2} electrode (24), so that small increments of the P_{ICO_2} may not cause measurable changes of the arterial P_{CO_2}. Sampling error can also distort the P_{CO_2} measurement in blood samples. Many physiological and environmental factors can alter the P_{CO_2} and pH of the blood and tissues. The level of muscular activity, diet, and ambient temperature are renowned modifiers of the actual CO_2 levels within a host. These are, in turn, influenced by prolonged confinement in crowded living conditions. The combined effects of confinement and atmosphere contaminants other than CO_2 (25) make it imperative to conduct well-controlled experiments permitting discrimination of CO_2 effects from all other effects.

POSITION OF NSMRL REGARDING EXPOSURE TO LOW-LEVEL CO_2

NSMRL has been actively involved with studying the responses of humans and animals to chronic, low-level, CO_2 exposures since the advent of the nuclear-powered submarine. Numerous patrol studies have provided a sizable data base of levels of CO_2 exposure and health problems encountered by Naval nuclear submariners (25,29). Humans have been exposed to a variety of concentrations of CO_2 for chronic periods of time aboard submarines, as well as in laboratory environments. Mean data, P_{ICO_2} = 10 torr and 40 days, collectively represent this wide variety of CO_2 exposures. Physiological responses to the CO_2 environment were repeatedly documented, but toxic effects were not apparent. Human exposures have been safely conducted in atmospheres containing up to 5 torr CO_2, for up to 90 days. Such exposures are therefore considered safe at this time.

The animal studies showed that chronic, low level, CO_2 exposures caused changes of the bone content of CO_2, the bone content of calcium, the soft tissue content of calcium, and histological changes of the lung (22,28). These changes were found to be completely reversible upon discontinuance of the animal's exposure to CO_2 (22,31). The extrapolation of the animal data to humans is a current problem in the advancement of our understanding of the effects of CO_2 on humans.

There are considerable technical and financial impediments to conducting fruitful investigations of the effects of chronic exposures to low-levels of CO_2. The accurate maintenance of the required concentrationsof CO_2 in the investigated environment is a costly endeavor. Likewise, the proper maintenance of a control environment (which would be in every way identical to the CO_2-environment except for the addition of CO_2) is costly. Many of the measurements of the organism's responses to the CO_2 are difficult and insensitive and therefore currently limit low-level CO_2 research efforts. There are also significant factors which can modify an organism's responses to low-level CO_2. Failure to control these factors may result in false interpretations of the data from the experiments.

Future, unforeseen developments may compel the U. S. Navy to continue its medical research into the health effects of CO_2. High-level CO_2 research would not be fraught

with as many technical limitations to the gathering of meaningful information as would research in low-level, CO_2 exposures. Given the appropriate facilities, manpower, and funding for research, the following categories represent potentially useful endeavors with regard to learning more about chronic, low-level CO_2:

a) Epidemiological studies of submariners should probe the possible toxic effects of CO_2;

b) Field studies of naval submariners should examine the effects of 90-day exposures (or longer) to CO_2. Attempts could be made to document the existence of increased stores of CO_2 in the body immediately following submarine patrols;

c) More sensitive tests of biochemical, bio-physical, and behavioral functions should be performed on man, and appropriate animals, during studies of the effects of residence in CO_2, and

d) Continued emphasis should be placed on the proper "controls" for CO_2 experiments. The goal is to discriminate the effects of CO_2 from all other factors which can modify the organism's CO_2 stores and general physiological function.

ADDENDA

Leitch and Smith wrote an excellent review of the literature on chronic human exposures to CO_2. Of particular relevance was their excellent discussion of the primary effects of CO_2 as related to submariners (33). The Undersea Medical Society published proceedings from a workshop on CO_2 contamination of the atmosphere (34). The emphasis of the conference was on the physiological and health effects of CO_2 in submarines. The information which was presented reinforced the position of this paper.

Other reports of human exposures to CO_2 were reviewed after the preparation of this manuscript (67-73). Of particular interest was the reported deterioration of health and work capacity at the beginning of the third month of human exposure to 0.8 to 1.8% CO_2. The report was in Russian, so that details await translation of the manuscript (67). The remaining references (68-73) confirmed the safety of low-level CO_2 exposures aboard Naval nuclear submarines.

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Unclassified

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER NSMRL Rpt# 973	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* NSMRL Position Paper: The Toxic Effects of Chronic Exposures to Low Levels of Carbon Dioxide		5. TYPE OF REPORT & PERIOD COVERED Interim Report 6. PERFORMING ORG. REPORT NUMBER NSMRL Rpt. #973
7. AUTHOR*(s)*		8. CONTRACT OR GRANT NUMBER*(s)*
9. PERFORMING ORGANIZATION NAME AND ADDRESS Naval Submarine Medical Research Laboratory Box 900, Naval Submarine Base New London Groton, CT 06349		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Naval Medical Research and Development Command National Naval Medical Center Bethesda, MD 20814		12. REPORT DATE 19 January 1982 13. NUMBER OF PAGES 37
14. MONITORING AGENCY NAME & ADDRESS*(if different from Controlling Office)*		15. SECURITY CLASS. *(of this report)* Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Carbon dioxide	Atmospheric contaminants
Exposure	Toxicity
Low level	Review
Submarine Medicine	Humans

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Numerous patrol studies have provided a sizable data base of levels of CO_2 exposure and health problems encountered by Naval nuclear submariners. Humans have been exposed to a variety of concentrations of CO_2 for chronic periods of time aboard submarines, as well as in laboratory environments. Mean data, P_{ICO_2} = 10 torr and 40 days, collectively represent this wide variety of CO_2 exposures. >Physiological responses to the CO_2 environment were repeatedly documented, but toxic effects were not apparent.< Human exposures were safely

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S/N 0102-014-6601 |

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